%RNA integrity in the context of the COVID-19 shots

Why is this important? Is it related to SAE reporting frequency?

Jessica Rose
Dec 6

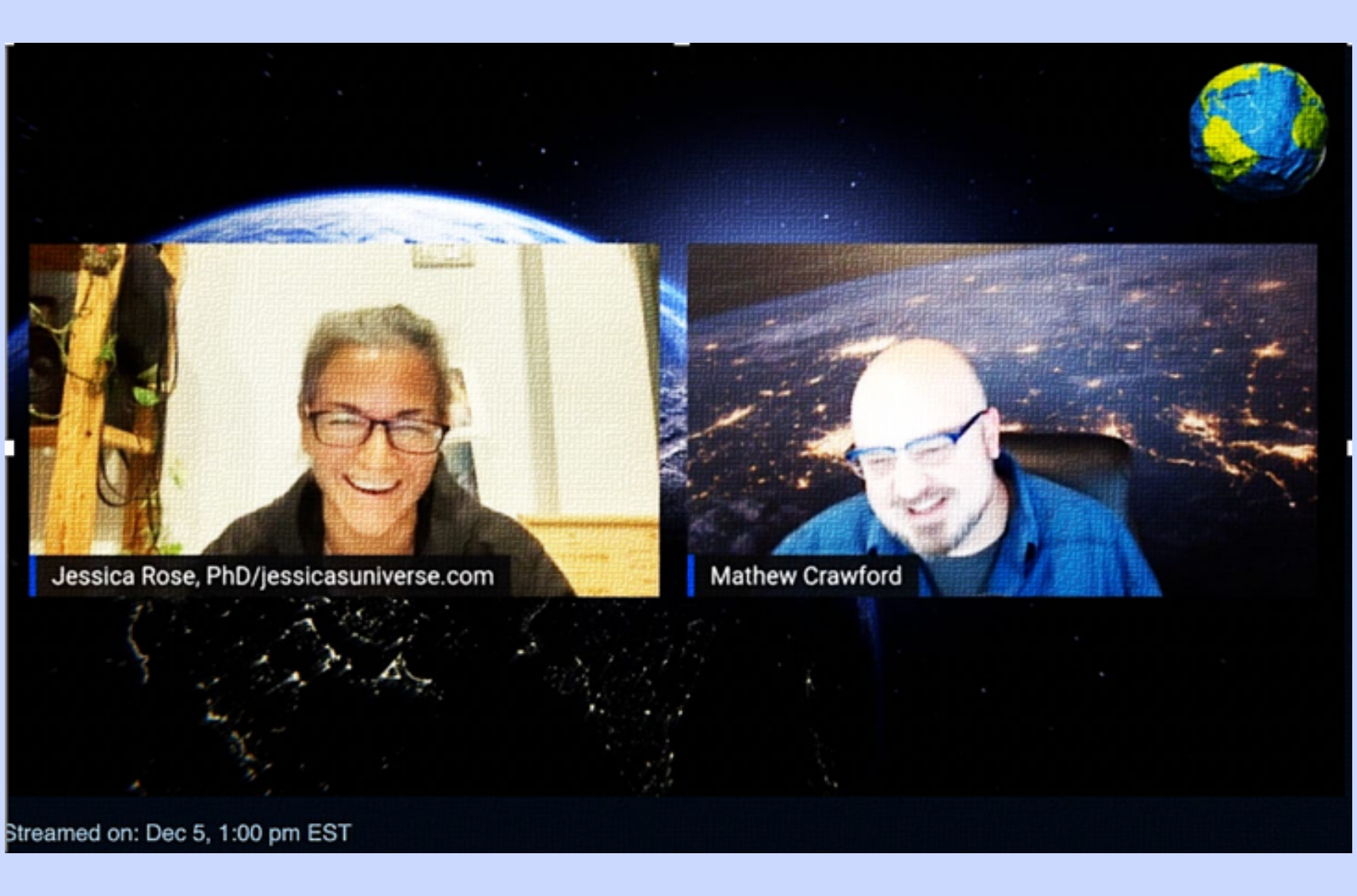
♡293 💬99

I had a chat with my dear buddy Mathew Crawford yesterday on his podcast Rounding the Earth. You can watch that here. As detail corrections, the spike protein is 1273 residues long for SARS-CoV-2. :) This is likely why Moderna refers to their product as mRNA-1273.

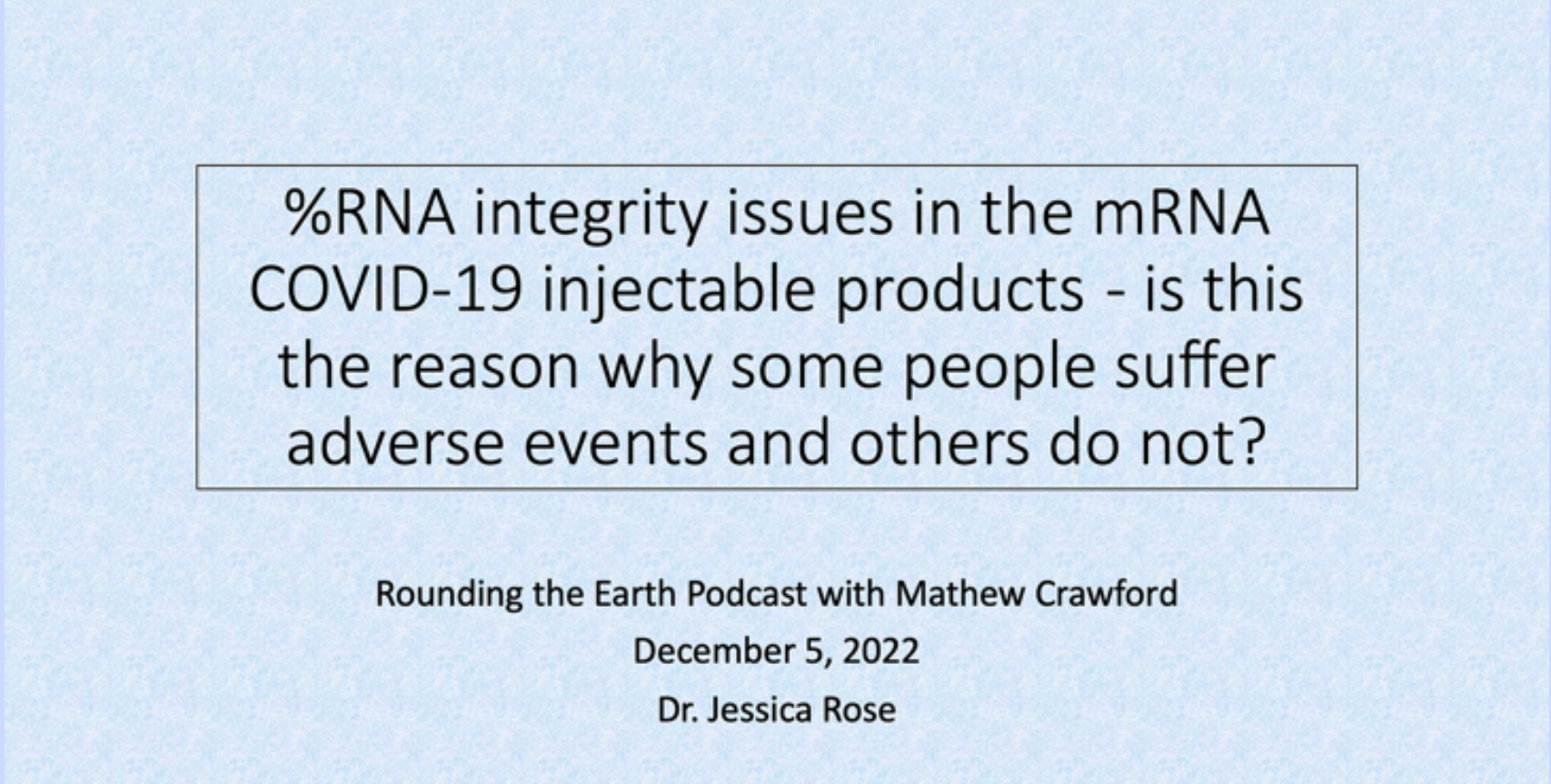
> The full-length of SARS-CoV-2 S is 1273 amino acid long with several functional domains and multiple proteolytic cleavage sites at S1/S2 boundary and S2' site.[1]

Also, at the end when we were doing our modeling exercise, I had read the number of doses per vax lot data wrong. It does not change the conclusion that there are 'order of magnitude' differences within the vax lots with respect to number of doses available. The following are the correct max and min values for number of doses per vax lot according to batch size and shipment numbers.

> Maximum number of doses = 1,539,300 (There were 15,393 shipments of vax lot FL0007 with 100 doses per shipment.)
>
> Minimum number of doses = 58,500 (There were 5 shipments of vax lot EP7533 with 11,700 doses per shipment.)

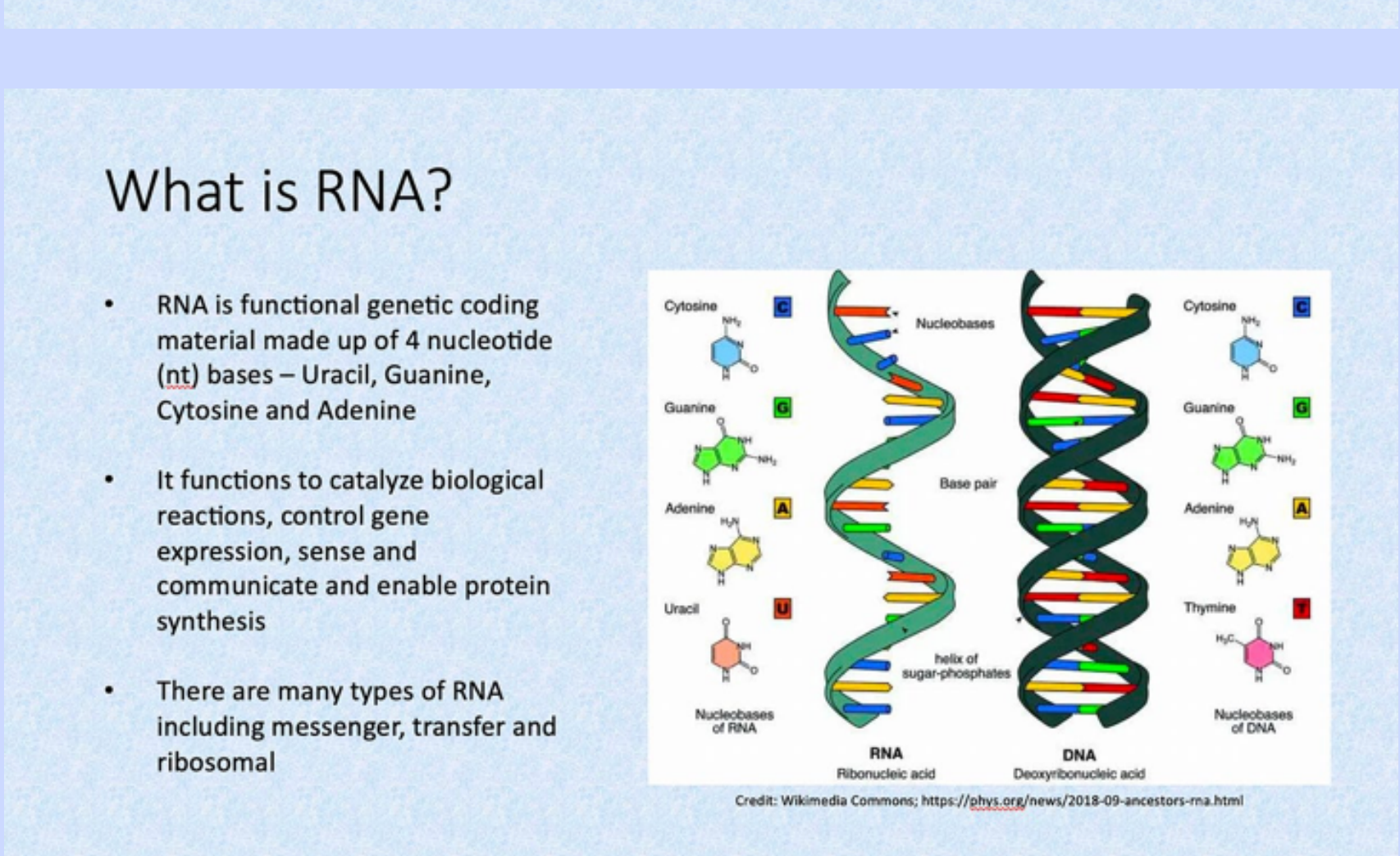

Jessica Rose, PhD/jessicasuniverse.com
Mathew Crawford
Streamed on: Dec 5, 1:00 pm EST

I actually prepared a slide deck for use in this podcast as sort of a 'lecture format', but I couldn't screen share so I will leave the slides here. :) The slide deck is a really back to basics Biochem 101 course combined with %RNA integrity data leaks and evidence that this known low %RNA integrity is causing immunological chaos.



%RNA integrity issues in the mRNA COVID-19 injectable products - is this the reason why some people suffer adverse events and others do not?

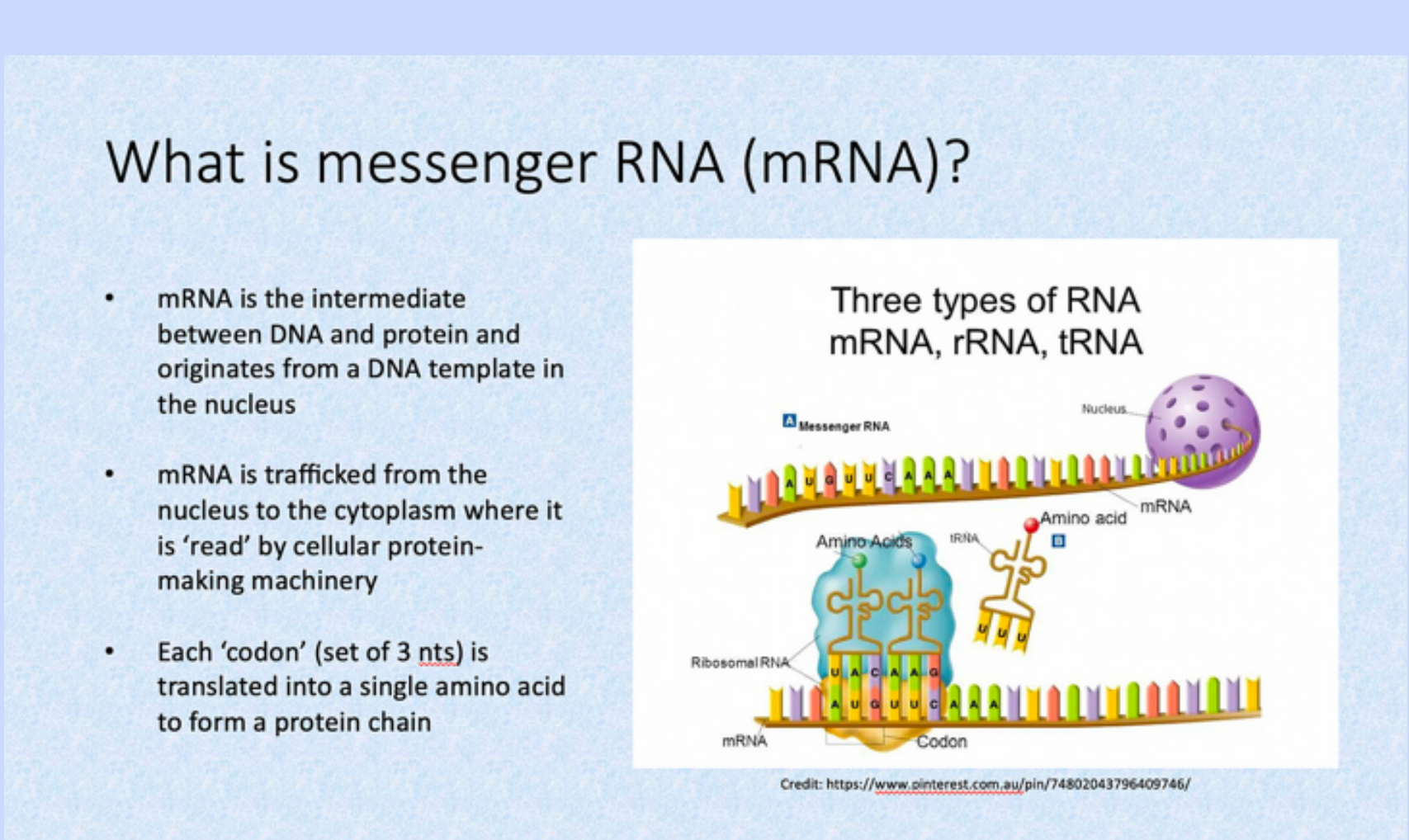
Rounding the Earth Podcast with Mathew Crawford
December 5, 2022
Dr. Jessica Rose



What is RNA?

- RNA is functional genetic coding material made up of 4 nucleotide (nt) bases – Uracil, Guanine, Cytosine and Adenine
- It functions to catalyze biological reactions, control gene expression, sense and communicate and enable protein synthesis
- There are many types of RNA including messenger, transfer and ribosomal

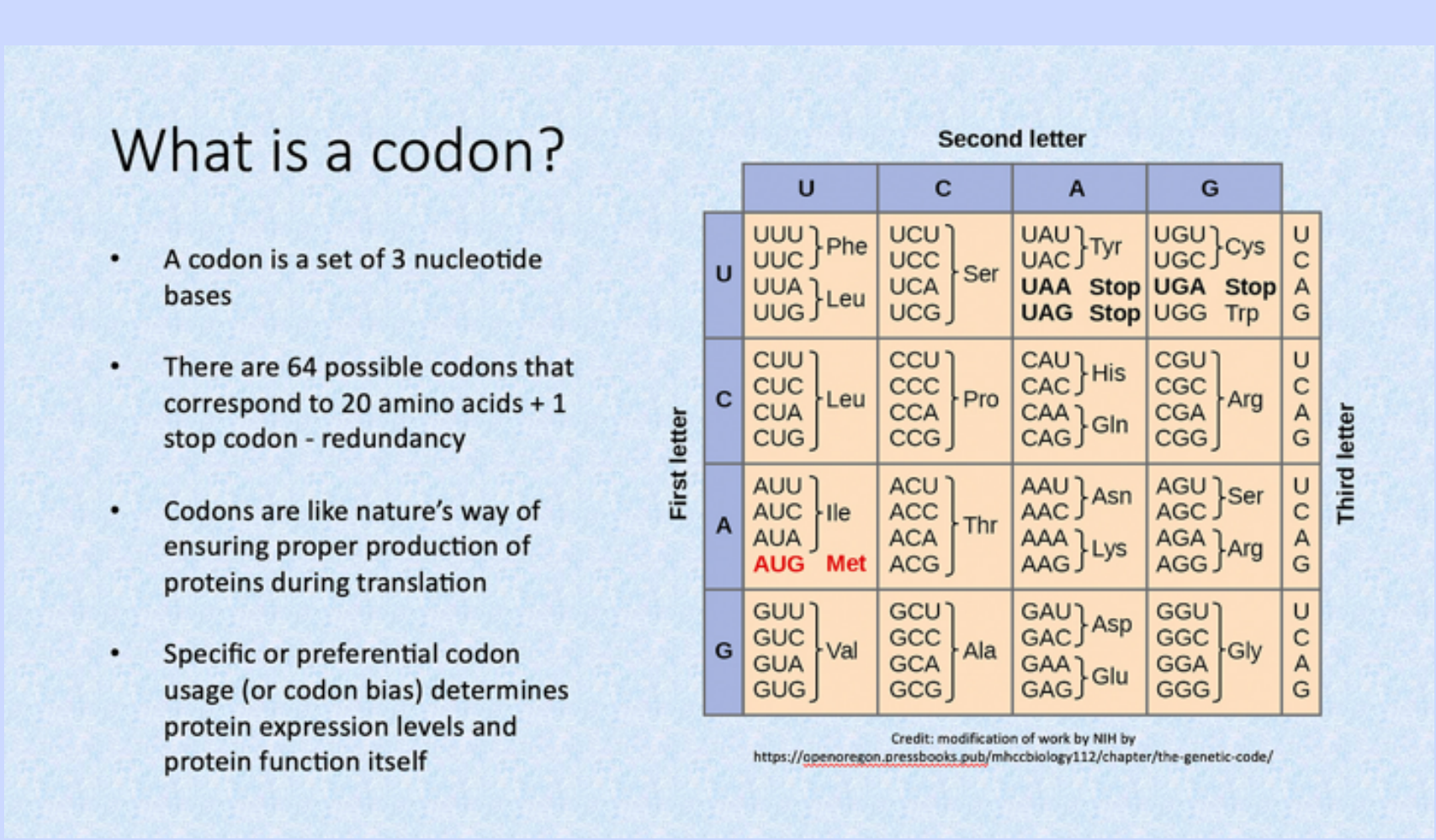
Credit: Wikimedia Commons; https://phys.org/news/2018-09-ancestors-rna.html



What is messenger RNA (mRNA)?

- mRNA is the intermediate between DNA and protein and originates from a DNA template in the nucleus
- mRNA is trafficked from the nucleus to the cytoplasm where it is 'read' by cellular protein-making machinery
- Each 'codon' (set of 3 nts) is translated into a single amino acid to form a protein chain

Credit: https://www.pinterest.com.au/pin/74802043796409746/



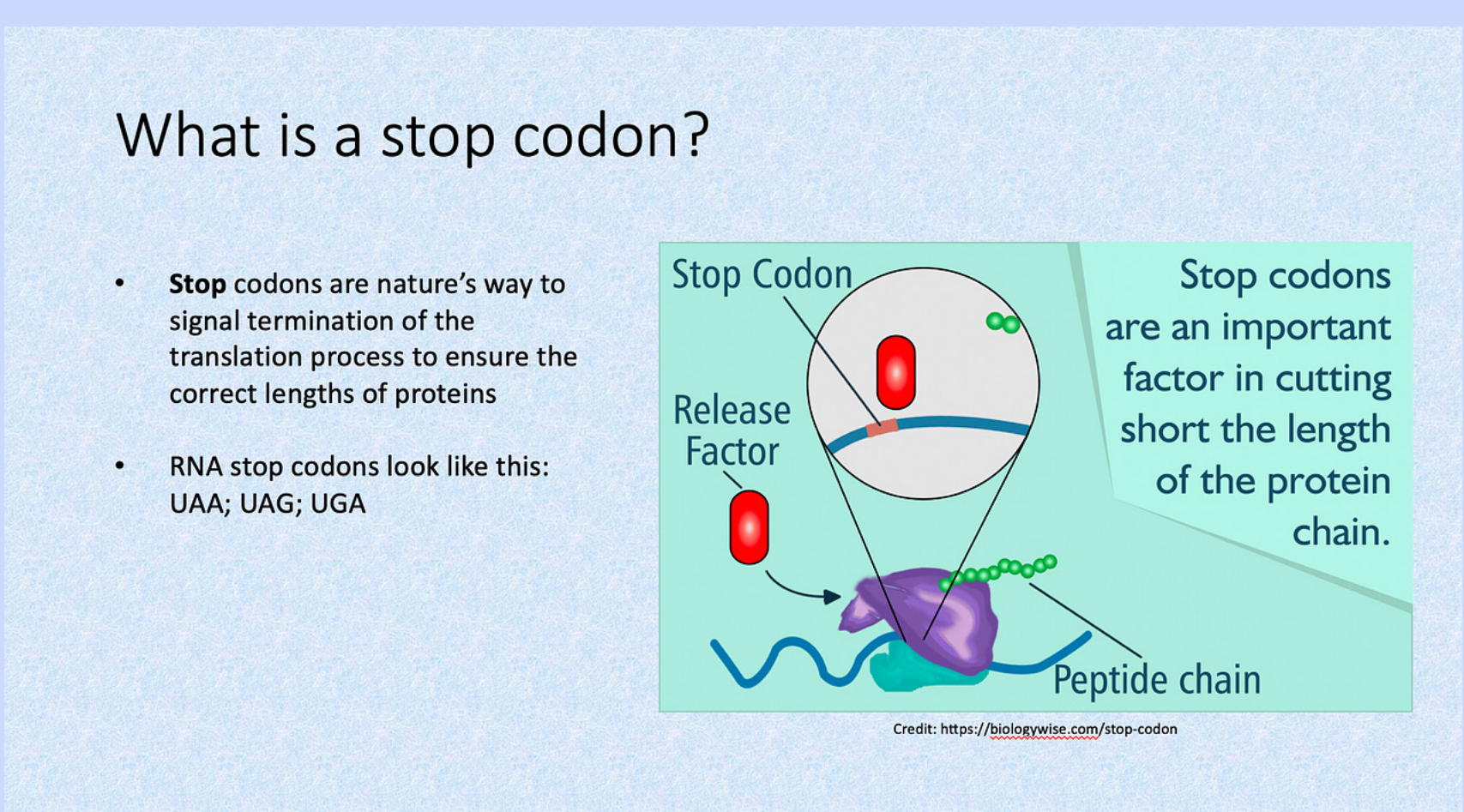
What is a codon?

- A codon is a set of 3 nucleotide bases
- There are 64 possible codons that correspond to 20 amino acids + 1 stop codon - redundancy
- Codons are like nature's way of ensuring proper production of proteins during translation
- Specific or preferential codon usage (or codon bias) determines protein expression levels and protein function itself

Second letter

First letter	U	C	A	G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr **UAA Stop** **UAG Stop**	UGU Cys UGC Cys **UGA Stop** UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Credit: modification of work by NIH by https://openoregon.pressbooks.pub/mhccbiology112/chapter/the-genetic-code/

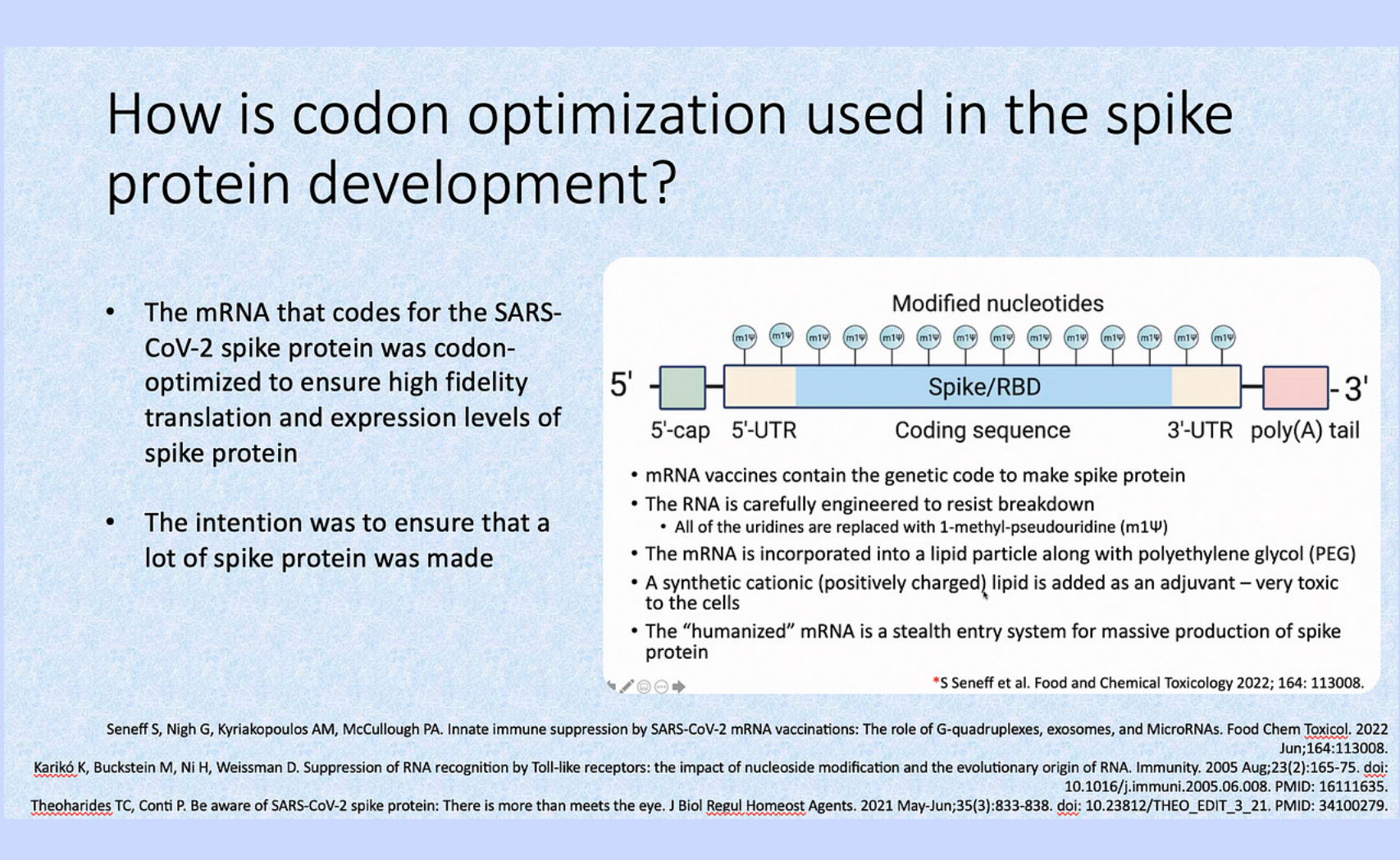


What is a stop codon?

- **Stop** codons are nature's way to signal termination of the translation process to ensure the correct lengths of proteins
- RNA stop codons look like this: UAA; UAG; UGA

Stop codons are an important factor in cutting short the length of the protein chain.

Credit: https://biologywise.com/stop-codon



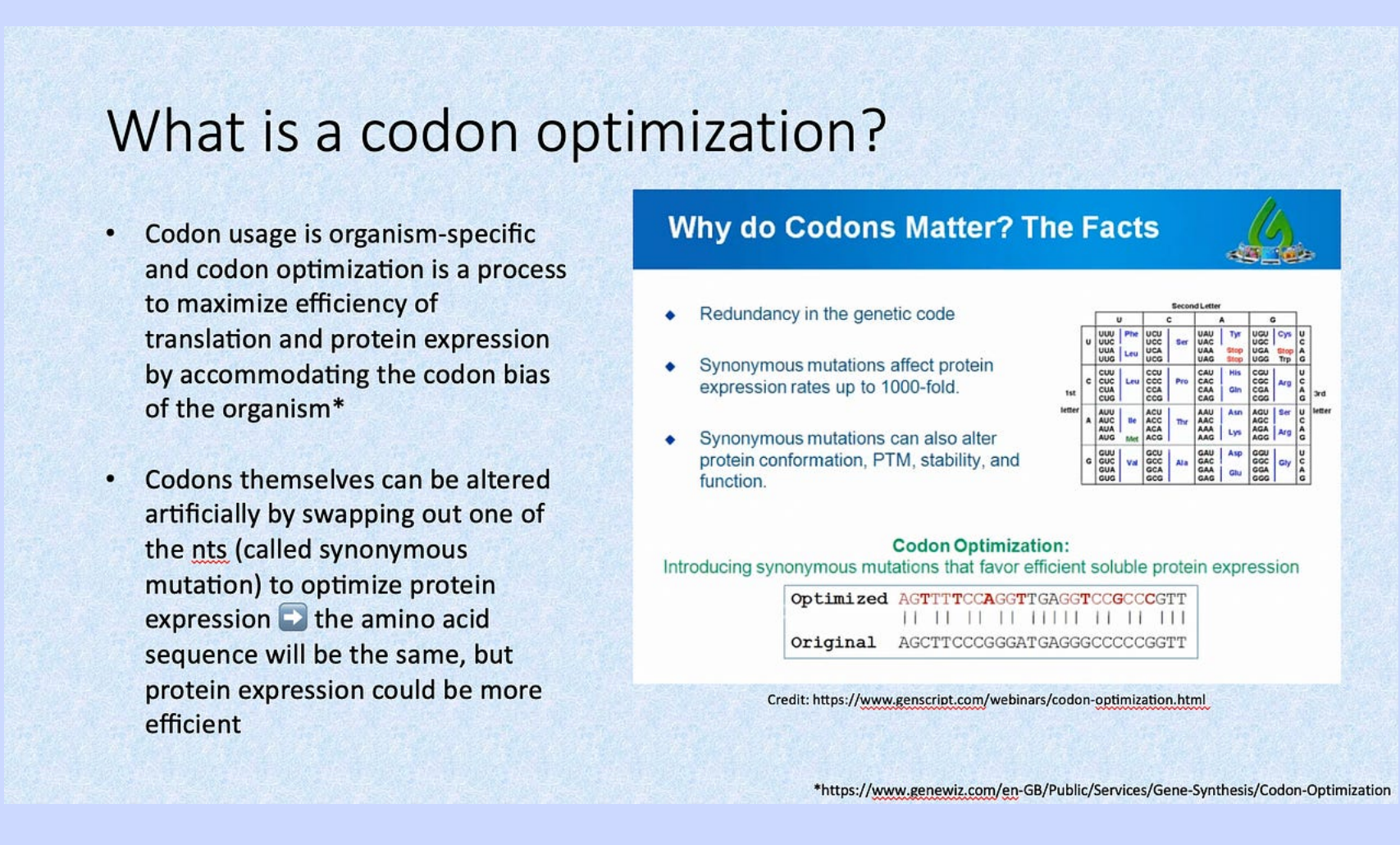
How is codon optimization used in the spike protein development?

- The mRNA that codes for the SARS-CoV-2 spike protein was codon-optimized to ensure high fidelity translation and expression levels of spike protein
- The intention was to ensure that a lot of spike protein was made

- mRNA vaccines contain the genetic code to make spike protein
- The RNA is carefully engineered to resist breakdown
  - All of the uridines are replaced with 1-methyl-pseudouridine (m1Ψ)
- The mRNA is incorporated into a lipid particle along with polyethylene glycol (PEG)
- A synthetic cationic (positively charged) lipid is added as an adjuvant – very toxic to the cells
- The "humanized" mRNA is a stealth entry system for massive production of spike protein

*S Seneff et al. Food and Chemical Toxicology 2022; 164: 113008.

Seneff S, Nigh G, Kyriakopoulos AM, McCullough PA. Innate immune suppression by SARS-CoV-2 mRNA vaccinations: The role of G-quadruplexes, exosomes, and MicroRNAs. Food Chem Toxicol. 2022 Jun;164:113008.
Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005 Aug;23(2):165-75. doi: 10.1016/j.immuni.2005.06.008. PMID: 16111635.
Theoharides TC, Conti P. Be aware of SARS-CoV-2 spike protein: There is more than meets the eye. J Biol Regul Homeost Agents. 2021 May-Jun;35(3):833-838. doi: 10.23812/THEO_EDIT_3_21. PMID: 34100279.



What is a codon optimization?

- Codon usage is organism-specific and codon optimization is a process to maximize efficiency of translation and protein expression by accommodating the codon bias of the organism*
- Codons themselves can be altered artificially by swapping out one of the nts (called synonymous mutation) to optimize protein expression ➡️ the amino acid sequence will be the same, but protein expression could be more efficient

Why do Codons Matter? The Facts

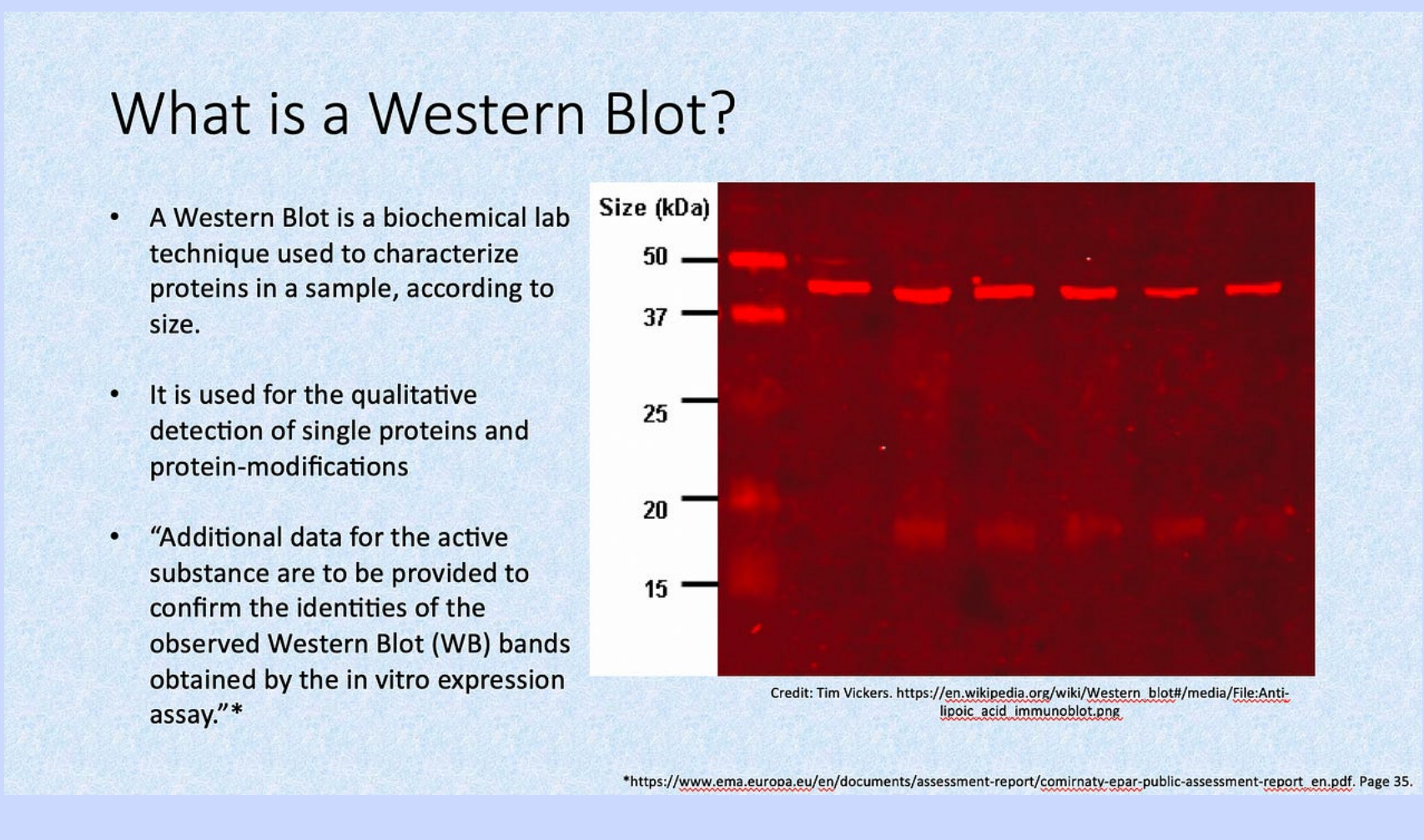
- Redundancy in the genetic code
- Synonymous mutations affect protein expression rates up to 1000-fold.
- Synonymous mutations can also alter protein conformation, PTM, stability, and function.

Codon Optimization:
Introducing synonymous mutations that favor efficient soluble protein expression

```
Optimized AGTTTTCCAGGTTGAGGTCCGCCCGTT
          || || || || ||||| || || |||
Original  AGCTTCCCGGGATGAGGGCCCCCGGTT
```

Credit: https://www.genscript.com/webinars/codon-optimization.html

*https://www.genewiz.com/en-GB/Public/Services/Gene-Synthesis/Codon-Optimization

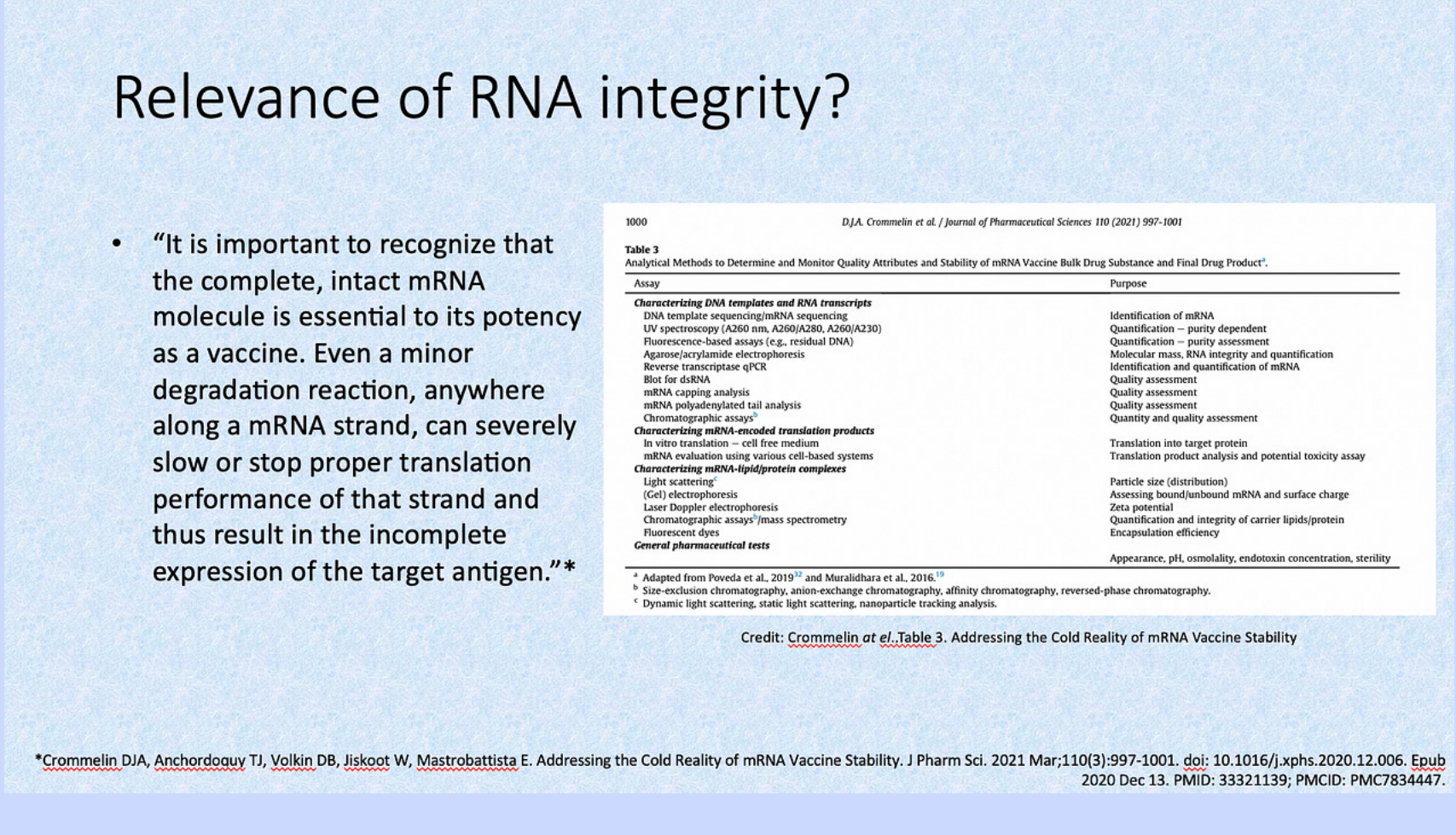


What is a Western Blot?

- A Western Blot is a biochemical lab technique used to characterize proteins in a sample, according to size.
- It is used for the qualitative detection of single proteins and protein-modifications
- "Additional data for the active substance are to be provided to confirm the identities of the observed Western Blot (WB) bands obtained by the in vitro expression assay."*

Credit: Tim Vickers. https://en.wikipedia.org/wiki/Western_blot#/media/File:Anti-lipoic_acid_immunoblot.png

*https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf. Page 35.



Relevance of RNA integrity?

- "It is important to recognize that the complete, intact mRNA molecule is essential to its potency as a vaccine. Even a minor degradation reaction, anywhere along a mRNA strand, can severely slow or stop proper translation performance of that strand and thus result in the incomplete expression of the target antigen."*

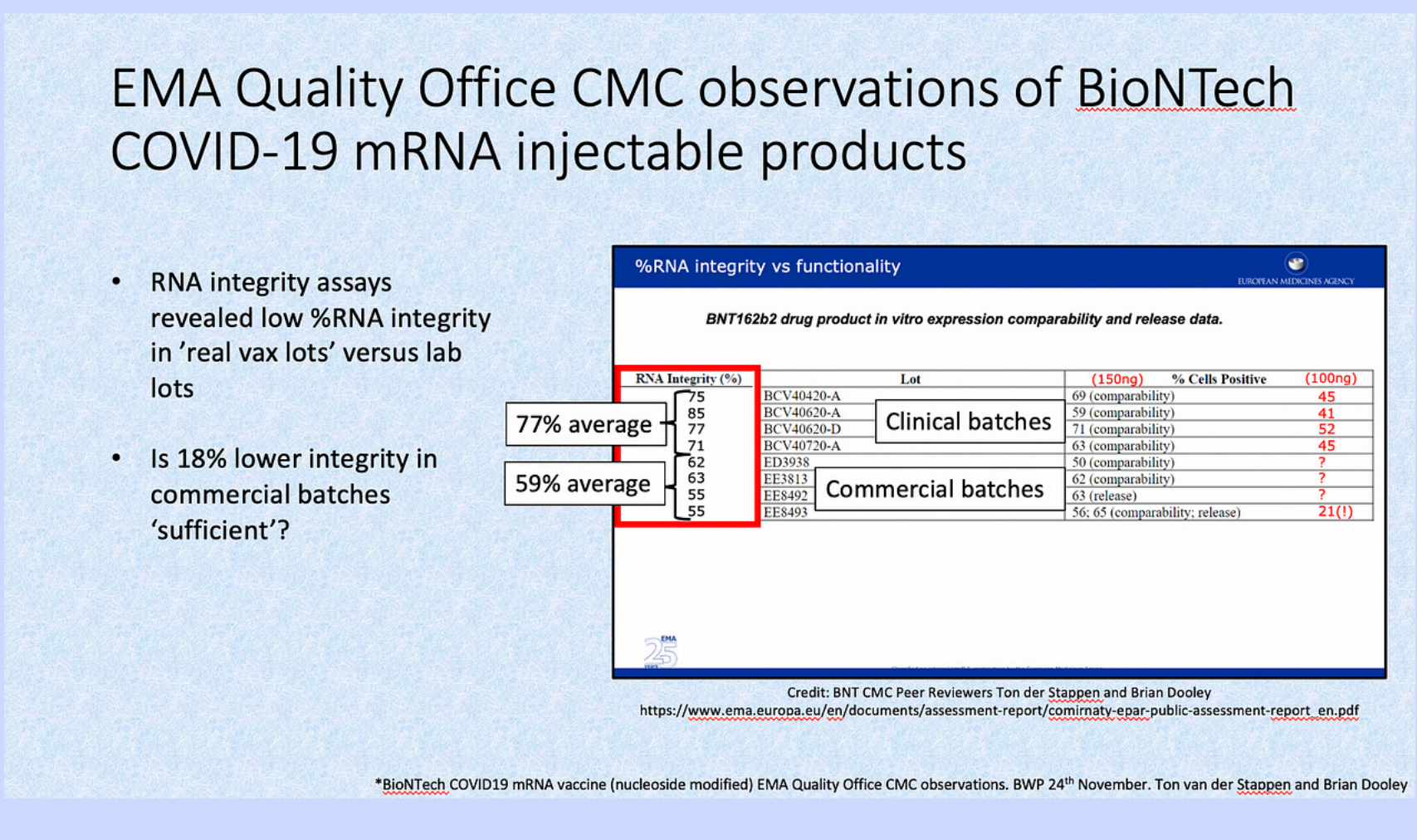
1000 D.J.A. Crommelin et al. / Journal of Pharmaceutical Sciences 110 (2021) 997-1001

Table 3
Analytical Methods to Determine and Monitor Quality Attributes and Stability of mRNA Vaccine Bulk Drug Substance and Final Drug Product

Assay	Purpose
Characterizing DNA templates and RNA transcripts	
DNA template sequencing/mRNA sequencing	Identification of mRNA
UV spectroscopy (A260 nm, A260/A280, A260/A230)	Quantification – purity dependent
Fluorescence-based assays (e.g., residual DNA)	Quantification – purity assessment
Agarose/acrylamide electrophoresis	Molecular mass, RNA integrity and quantification
Reverse transcriptase qPCR	Identification and quantification of mRNA
Blot for dsRNA	Quality assessment
mRNA capping analysis	Quality assessment
mRNA polyadenylated tail analysis	Quality assessment
Chromatographic assays	Quantity and quality assessment
Characterizing mRNA-encoded translation products	
In vitro translation – cell free medium	Translation into target protein
mRNA evaluation using various cell-based systems	Translation product analysis and potential toxicity assay
Characterizing mRNA-lipid/protein complexes	
Light scattering	Particle size (distribution)
(Gel) electrophoresis	Assessing bound/unbound mRNA and surface charge
Laser Doppler electrophoresis	Zeta potential
Chromatographic assays/mass spectrometry	Quantification and integrity of carrier lipids/protein
Fluorescent dyes	Encapsulation efficiency
General pharmaceutical tests	Appearance, pH, osmolality, endotoxin concentration, sterility

Credit: Crommelin et al. Table 3. Addressing the Cold Reality of mRNA Vaccine Stability

*Crommelin DJA, Anchordoquy TJ, Volkin DB, Jiskoot W, Mastrobattista E. Addressing the Cold Reality of mRNA Vaccine Stability. J Pharm Sci. 2021 Mar;110(3):997-1001. doi: 10.1016/j.xphs.2020.12.006. Epub 2020 Dec 13. PMID: 33321139; PMCID: PMC7834447.



EMA Quality Office CMC observations of BioNTech COVID-19 mRNA injectable products

- RNA integrity assays revealed low %RNA integrity in 'real vax lots' versus lab lots
- Is 18% lower integrity in commercial batches 'sufficient'?

%RNA integrity vs functionality

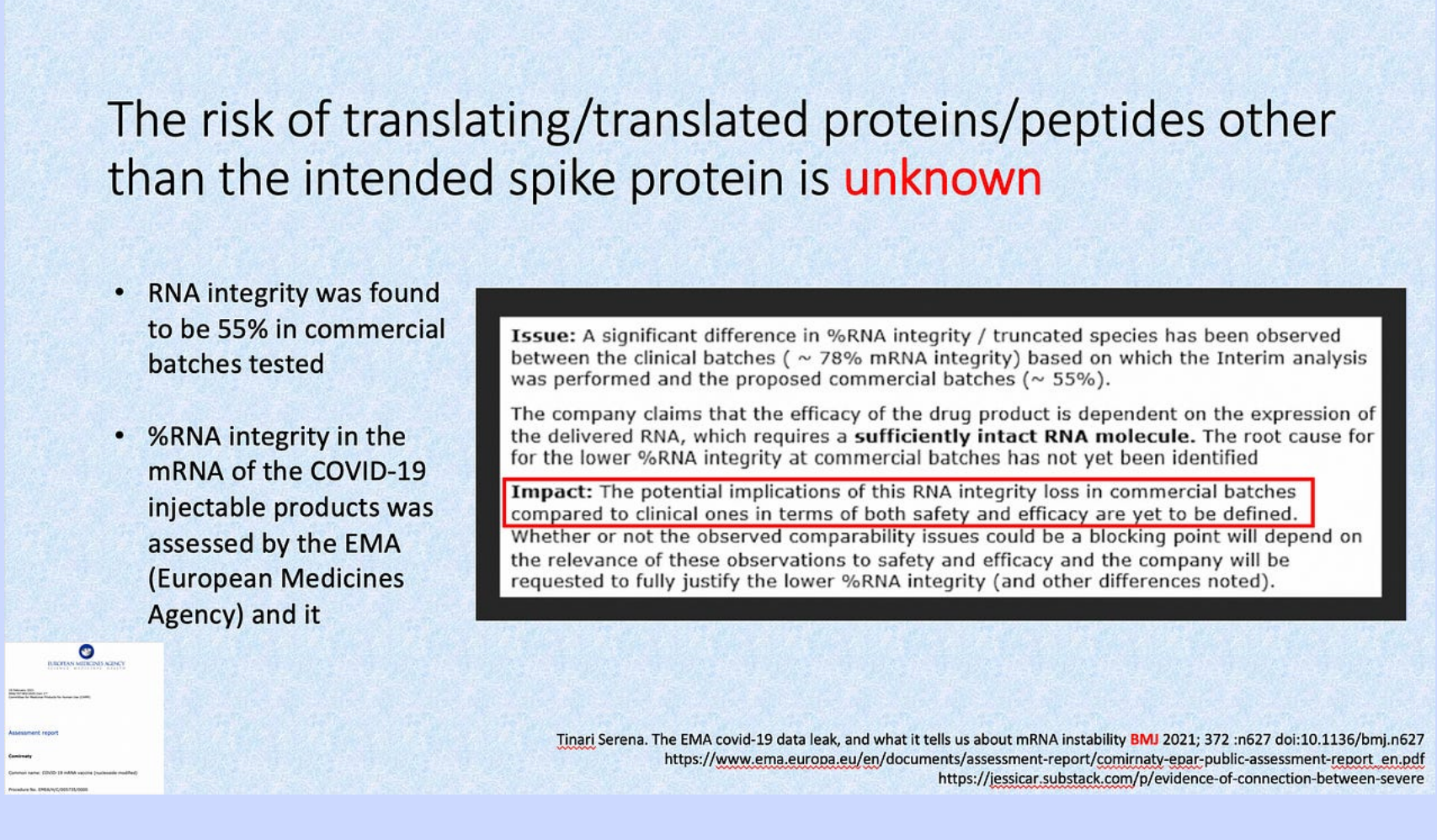
BNT162b2 drug product in vitro expression comparability and release data.

RNA Integrity (%)	Lot	% Cells Positive (150ng)	% Cells Positive (100ng)
75	BCV40420-A	69 (comparability)	45
85	BCV40620-A	59 (comparability)	41
77	BCV40620-D	71 (comparability)	52
71	BCV40720-A	63 (comparability)	45
62	ED3938	50 (comparability)	?
63	EE3813	62 (comparability)	?
55	EE8492	63 (release)	?
55	EE8493	56; 65 (comparability; release)	21(!)

77% average — Clinical batches
59% average — Commercial batches

Credit: BNT CMC Peer Reviewers Ton der Stappen and Brian Dooley
https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

*BioNTech COVID19 mRNA vaccine (nucleoside modified) EMA Quality Office CMC observations. BWP 24th November. Ton van der Stappen and Brian Dooley



The risk of translating/translated proteins/peptides other than the intended spike protein is unknown

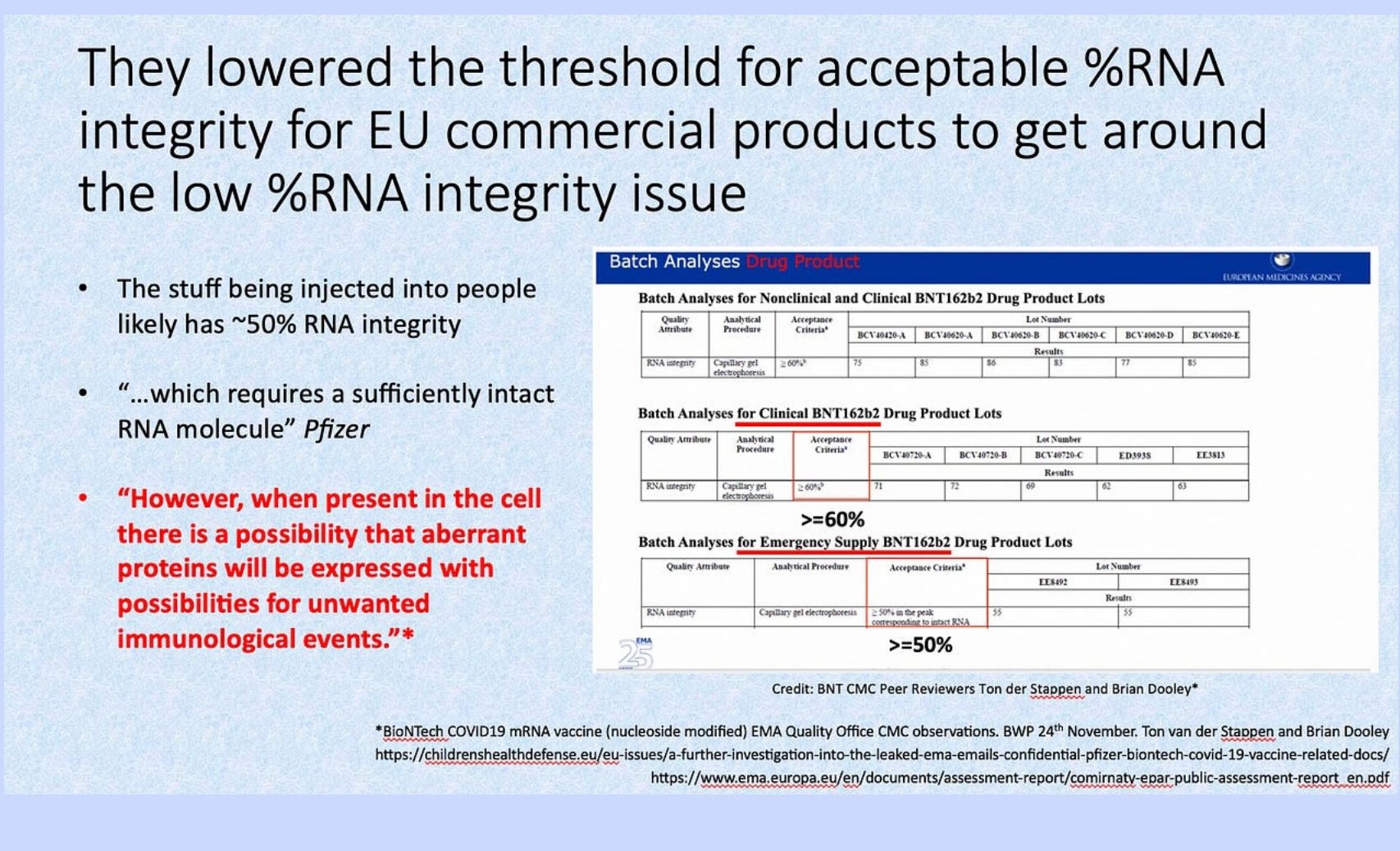
- RNA integrity was found to be 55% in commercial batches tested
- %RNA integrity in the mRNA of the COVID-19 injectable products was assessed by the EMA (European Medicines Agency) and it

Issue: A significant difference in %RNA integrity / truncated species has been observed between the clinical batches (~ 78% mRNA integrity) based on which the Interim analysis was performed and the proposed commercial batches (~ 55%).

The company claims that the efficacy of the drug product is dependent on the expression of the delivered RNA, which requires a **sufficiently intact RNA molecule.** The root cause for for the lower %RNA integrity at commercial batches has not yet been identified

Impact: The potential implications of this RNA integrity loss in commercial batches compared to clinical ones in terms of both safety and efficacy are yet to be defined. Whether or not the observed comparability issues could be a blocking point will depend on the relevance of these observations to safety and efficacy and the company will be requested to fully justify the lower %RNA integrity (and other differences noted).

Tinari Serena. The EMA covid-19 data leak, and what it tells us about mRNA instability BMJ 2021; 372 :n627 doi:10.1136/bmj.n627
https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf
https://jessicar.substack.com/p/evidence-of-connection-between-severe



They lowered the threshold for acceptable %RNA integrity for EU commercial products to get around the low %RNA integrity issue

- The stuff being injected into people likely has ~50% RNA integrity
- "…which requires a sufficiently intact RNA molecule" *Pfizer*
- **"However, when present in the cell there is a possibility that aberrant proteins will be expressed with possibilities for unwanted immunological events."***

Batch Analyses Drug Product

Batch Analyses for Nonclinical and Clinical BNT162b2 Drug Product Lots

Batch Analyses for Clinical BNT162b2 Drug Product Lots

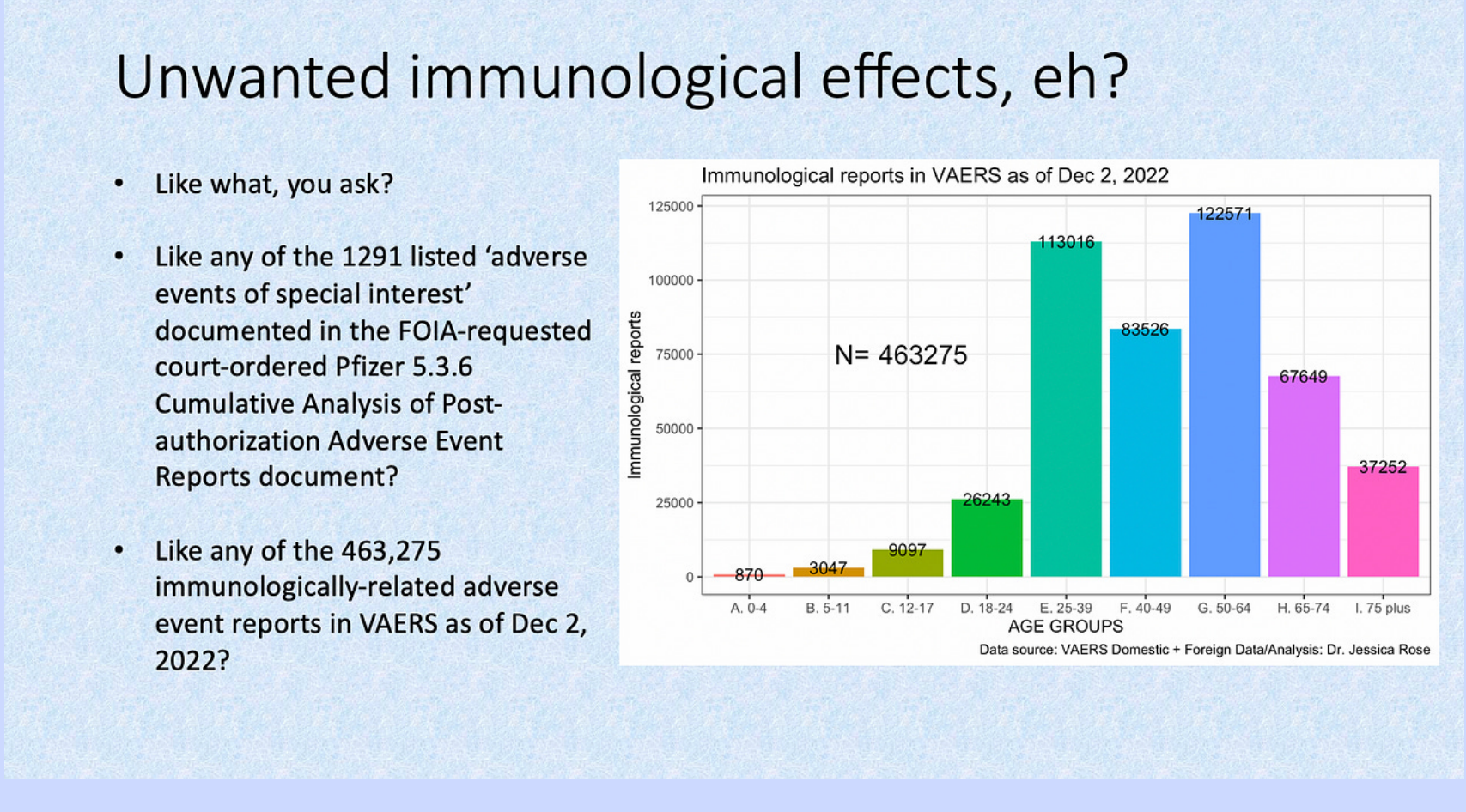
>=60%

Batch Analyses for Emergency Supply BNT162b2 Drug Product Lots

>=50%

Credit: BNT CMC Peer Reviewers Ton der Stappen and Brian Dooley*

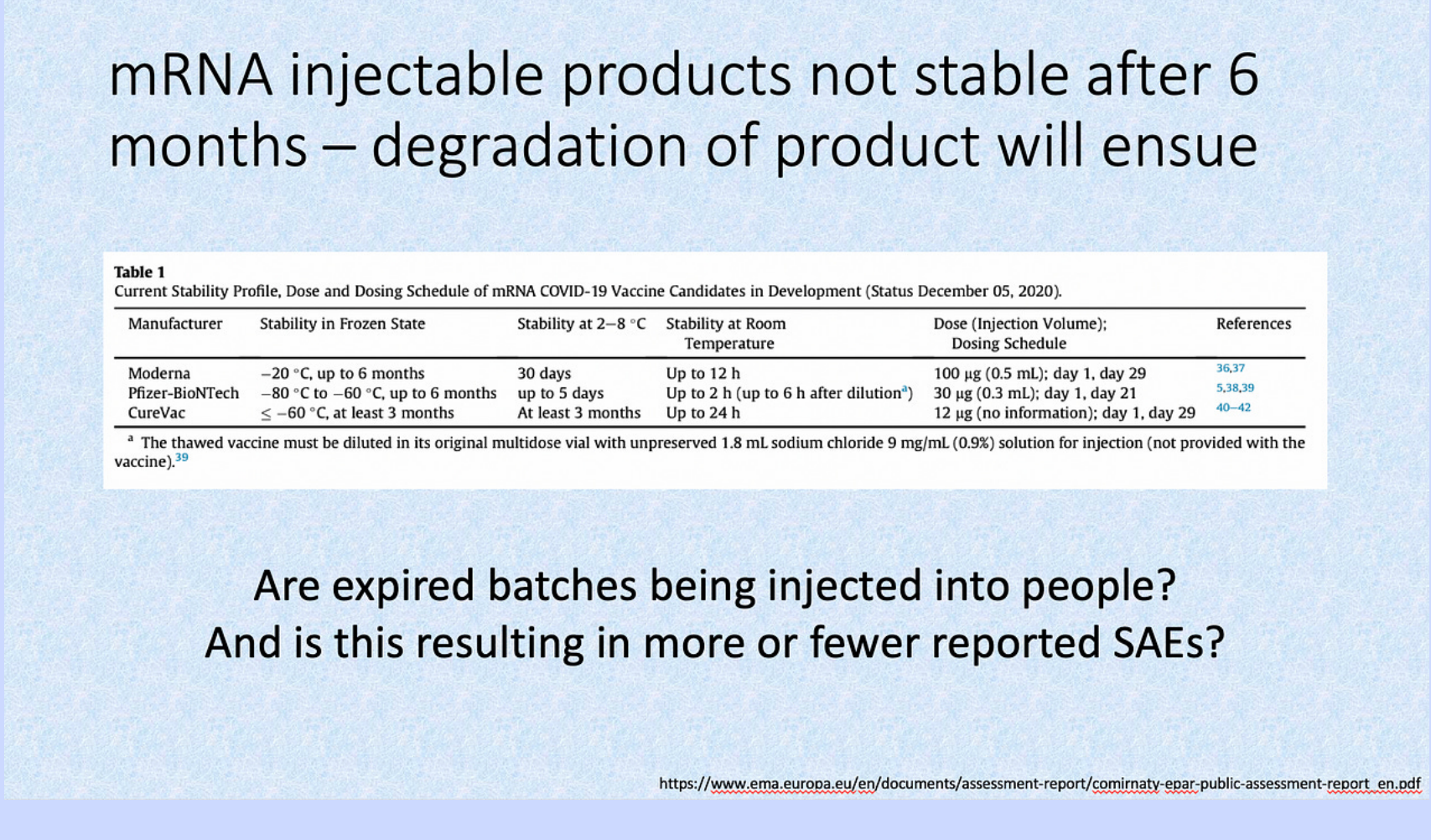
*BioNTech COVID19 mRNA vaccine (nucleoside modified) EMA Quality Office CMC observations. BWP 24th November. Ton van der Stappen and Brian Dooley
https://childrenshealthdefense.eu/eu-issues/a-further-investigation-into-the-leaked-ema-emails-confidential-pfizer-biontech-covid-19-vaccine-related-docs/
https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf



Unwanted immunological effects, eh?

- Like what, you ask?
- Like any of the 1291 listed 'adverse events of special interest' documented in the FOIA-requested court-ordered Pfizer 5.3.6 Cumulative Analysis of Post-authorization Adverse Event Reports document?
- Like any of the 463,275 immunologically-related adverse event reports in VAERS as of Dec 2, 2022?

Data source: VAERS Domestic + Foreign Data/Analysis: Dr. Jessica Rose



mRNA injectable products not stable after 6 months – degradation of product will ensue

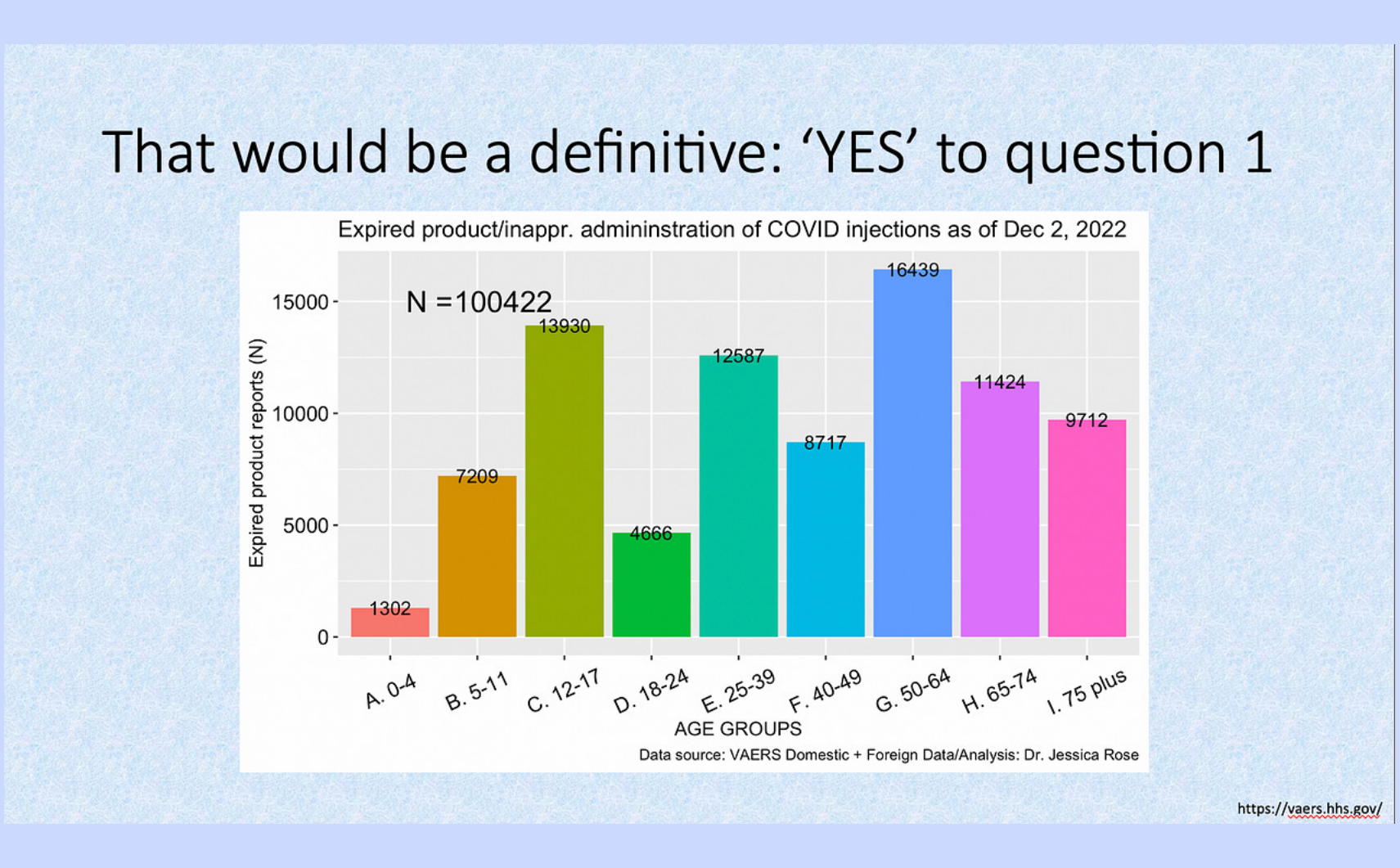
Table 1
Current Stability Profile, Dose and Dosing Schedule of mRNA COVID-19 Vaccine Candidates in Development (Status December 05, 2020).

Manufacturer	Stability in Frozen State	Stability at 2–8 °C	Stability at Room Temperature	Dose (Injection Volume); Dosing Schedule	References
Moderna	−20 °C, up to 6 months	30 days	Up to 12 h	100 µg (0.5 mL); day 1, day 29	36,37
Pfizer-BioNTech	−80 °C to −60 °C, up to 6 months	up to 5 days	Up to 2 h (up to 6 h after dilution[a])	30 µg (0.3 mL); day 1, day 21	5,38,39
CureVac	≤ −60 °C, at least 3 months	At least 3 months	Up to 24 h	12 µg (no information); day 1, day 29	40–42

[a] The thawed vaccine must be diluted in its original multidose vial with unpreserved 1.8 mL sodium chloride 9 mg/mL (0.9%) solution for injection (not provided with the vaccine).[39]

Are expired batches being injected into people?
And is this resulting in more or fewer reported SAEs?

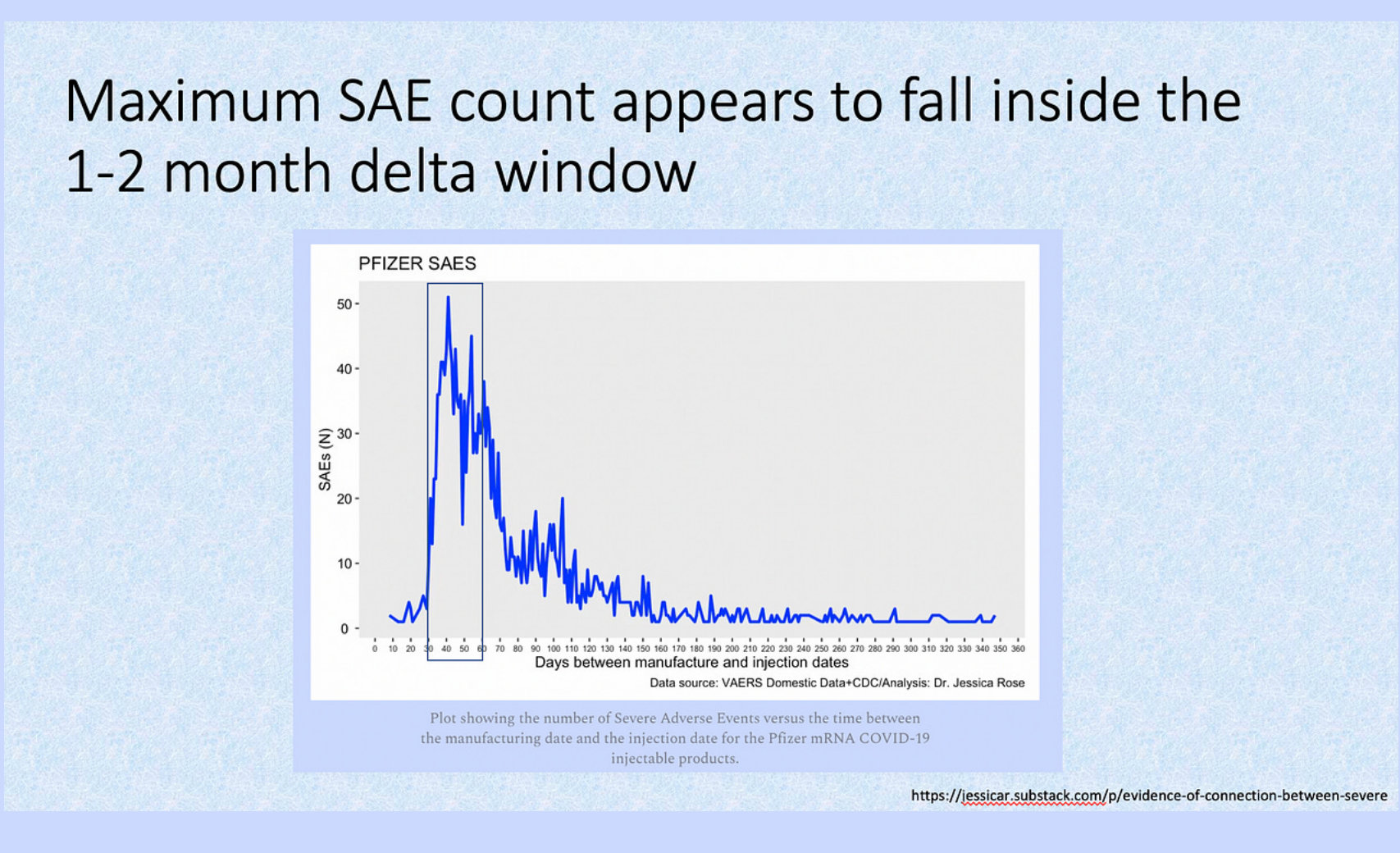
https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf



That would be a definitive: 'YES' to question 1

Data source: VAERS Domestic + Foreign Data/Analysis: Dr. Jessica Rose

https://vaers.hhs.gov/



Maximum SAE count appears to fall inside the 1-2 month delta window

Data source: VAERS Domestic Data+CDC/Analysis: Dr. Jessica Rose

Plot showing the number of Severe Adverse Events versus the time between the manufacturing date and the injection date for the Pfizer mRNA COVID-19 injectable products.

https://jessicar.substack.com/p/evidence-of-connection-between-severe

It may be that 'fresher' products do more damage and it may in turn be that this is the result of less degradation having ensued, ie: higher %RNA integrity → closer to full-length spike protein. Not sure yet.

I am currently looking into our unanswered questions concerning the effect of stratification by demographics, for example, and other neat stuff.

If you have cool ideas, pitch them in the comments. I will leave them open to all. :)

Stay tuned.

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[1] Zhu, C., He, G., Yin, Q., Zeng, L., Ye, X., Shi, Y., & Xu, W. (2021). Molecular biology of the SARs-CoV-2 spike protein: A review of current knowledge. *Journal of medical virology*, *93*(10), 5729–5741. https://doi.org/10.1002/jmv.27132.

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99 Comments

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Edward Flynn Writes Edward's Newsletter Dec 6 Liked by Jessica Rose

I listened to the RFKJR interview with Sasha Latypova and surmise that poor quality control by DOD's contractors is the reason anybody is still alive out of 80% "jabbed" in the US (or whatever it is).

The ability to adhere a refrigerator magnet to one's forehead seems to be an interesting side effect for a few. Might come in handy if you don't mind the reason for it. I get queasy thinking about it.

Aside from that, the Society of Actuaries report cited by Ed Dowd suggests a connection between COVID shot outcomes and the cumulative effect of assault by childhood vaccines. My question is posed below in a comment posted on Igor's sub stack. Just asking!

Thanks!

Prior post copied here:

The Society of Actuarials report posted by @Edward Dowd on GETTR shows a 100% spike in excess death for age 35 to 44 in Q3 '21. Age 35 was birth year 1986. This group effectively captures the escalation of childhood vaccines driven by the Vaccine Injury Compensation Act. This group was subsequently mandated by their employers when their busy boxes were replaced by laptops. Chronic immune suppression carried into adulthood that reacted in a catastrophic way to the COVID shots is a possible link to the enhanced vulnerability of younger folks to sudden death post vax. It tells of the inadequacy of all vaccine safety testing, the need to strip away legal Immunity from the manufacturers and to codify philosophical exemption to all vaccine requirements.

♡ 36 Reply Gift a subscription Collapse •••

1 reply

Katherine MacGilchrist Dec 6 Liked by Jessica Rose

Hi Jessica, thanks for all your writing and analysis. In UK we noticed that calls to ambulances for collapse/syncope and cardiac arrest sky rocketed shortly after the regulator here changed the thawed shelf life of the Pfizer injection from 5 days to 30 days. That was the from about 21st May 2021. I understand that the FDA did the same in the USA at about the same time. I wonder how this longer thawed shelf life will affect RNA integrity and whether this is linked to the SAEs. It would be interesting to look at pre-May 2021 vs post-May 2021 for Pfizer. Ideally one would want to plot time since thawing against SAE frequency but I guess that data isn't available.

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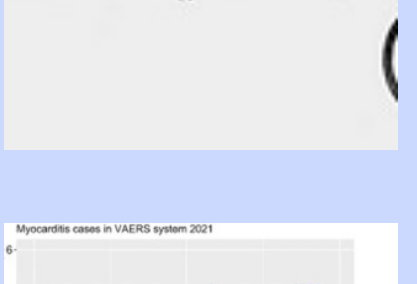
1 reply

97 more comments…

Top New Community

This is one of the emails I received the other day. I get hundreds daily, and I am hearing you all.
This particular note spoke loudly to me and this lovely person gave me permission to share her words.
JESSICA ROSE JUL 17 ♡1,614 💬230



A Report on Myocarditis Adverse Events in the U.S. Vaccine Adverse Events Reporting System (VAERS) in Association with COVID-19 Injectable…
Jessica Rose PhD, MSc, BSc and Peter A. McCullough MD, MPH
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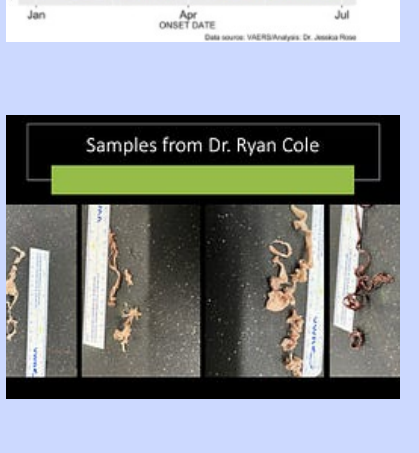

Rewrite: Let's tag team this until everybody understands
The modified spike protein is dangerous and for very specific reasons.
JESSICA ROSE JUN 13 ♡667 💬141

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